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Synthesis of Enantiomeric α-Cephalins[1,2]

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A method for the synthesis of both the enantiomeric and the racemic forms of fully saturated α-cephalins of assured constitutional and configurational purity has been developed and has been applied to the preparation of three homologous α-cephalins of the L-series, namely, distearoyl-, dipalmitoyl- and dimyristoylcephalin. The X-ray diffraction patterns, infrared absorption spectra, solubility in various organic solvents, and other physical data of the pure, individual, crystalline cephalins are reported.

Attempts to isolate individual cephalins (phosphatidyl ethanolamines) from biological sources have met with scant success. Levene and West,[4] fractionating a catalytically hydrogenated mixture of lecithin and cephalin (egg yolk) were the first to obtain a substance ($[\alpha]_D$ +6°) in which the nitrogen was present predominantly (93 and 97%) in the form of amino-nitrogen, and which seemed to possess the composition required by theory for distearoylcephalin (calcd. C, 65.82; H, 11.04; N, 1.87; P, 4.14. Found: C, 65.33; H, 11.56; N, 1.94; P, 3.87). In view of later developments, however, it was unfortunate that Levene and West failed to distinguish between amino-alcohol and amino acid-nitrogen, and thus left some doubt concerning the identity of their substance. Several years later Nishimoto and Suzuki[5] claimed to have obtained the distearoyl-α-cephalin from human brain (m.p. 175°). Subsequent investigations by Folch,[6-9] Wooley,[8,10] Malkin[11] and his associates disclosed that Thudichum's oxbrain "cephalin fraction," as well as "cephalin fractions" of plant origin, consists only partially of phosphatidyl ethanolamine, and that associated with it are variable amounts of phosphatidyl serine and phosphoric acid esters containing inositol, galactose, and as yet unidentified nitrogenous constituents. The phosphatidyl ethanolamine obtained by Folch[6] was partially unsaturated and seemed reasonably free of other phosphatides. To the authors' knowledge the foregoing summarizes the more successful attempts to isolate individual natural cephalins.

A study of the biological role of the various components of the "cephalin fractions" requires accessibility to the pure substances. The difficulties encountered in isolating pure individual cephalins (phosphatidyl ethanolamines) from natural sources have prompted several attempts to obtain these substances synthetically. The synthesis of pure individual β-cephalins has been accomplished finally by Rose,[12] and Hunter, Roberts and Kester.[13] None of the earlier attempts, however, to synthesize α-cephalins[14,15] has been successful. The authors herein report a procedure which is generally applicable to the synthesis of the racemic form, as well as to both enantiomeric forms of fully saturated α-cephalins. All reactions, including those for the preparation of the enantiomeric α,β-diglycerides, are such that the asymmetry of the carbon atoms 2 and 5 of D- and L-mannitol, the starting materials, is maintained throughout the synthesis, thus assuring the stereochemical purity of the α-cephalins. To prevent the formation of amides of phosphoric acid we followed Rose[12] and used (N-carbobenzoxy)-ethanolamine. The synthesis, briefly, is as follows: An α,β-diglyceride (I) in its D-, L- or racemic form is phosphorylated with phenylphosphoryl dichloride in the presence of 2 moles of pyridine, giving rise to the formation of diacyl α-glycerylphenylphosphoryl chloride (II) and bis-(diacylglyceryl)-phenylphosphate (III). The main reaction product (II), without separation from III, is immediately esterified with N-carbobenzoxyethanolamine in the presence of a large excess of pyridine. The reaction mixture is brought to dryness *in vacuo*, the diacyl α-glycerylphenylphosphoryl-(N-carbobenzoxy)-ethanolamine (IV) is extracted with petroleum ether and is freed from bis-α,α-(diacylglyceryl)-phenylphosphate (III)[16] by treatment

(1) Presented before the Chemical Institute of Canada, Toronto, June, 1950. A preliminary report of the subject matter of this paper has appeared in *Science*, **113**, 12 (1951).

(2) Although there is some confusion as to the particular chemical substance intended when the term cephalin is used, in this communication the term is applied in the sense conventionally used in text-books of chemistry and biochemistry, *i.e.*, to a series of homologous diacylglycerylphosphoryl ethanolamines (reaction scheme, structural formula V and VI) for which Folch has proposed the term phosphatidyl ethanolamine. Much of the present confusion could be avoided if Thudichum's "cephalin" were called "cephalin fraction" indicating its composite nature, and if the generic term cephalin were reserved for those compounds having the structure customarily associated with the cephalin molecule (V, VI). Newly discovered components of Thudichum's "cephalin fraction," not possessing the cephalin structure, could then be given new names, *e.g.*, phosphatidyl serine. The suggestion of retaining for the diacylglycerylphosphoryl ethanolamines the name cephalin is in line with the recent use of this term by Rose[12] and Hunter, Roberts and Kester.[13]

(3) Part of this paper forms a thesis submitted by Miss Margaret Russell to the Department of Biochemistry, University of Toronto, in partial fulfillment of the requirements for the degree of Bachelor of Arts, 1949.

(4) P. A. Levene and C. J. West, *J. Biol. Chem.*, **35**, 285 (1918).

(5) U. Nishimoto and B. Suzuki, *Proc. Imp. Acad.* [*Tokyo*], **8**, 424 (1932).

(6) J. Folch, *J. Biol. Chem.*, **146**, 35 (1942).

(7) J. Folch and H. A. Schneider, *ibid.*, **137**, 51 (1941).

(8) J. Folch and D. W. Wooley, *ibid.*, **142**, 963 (1942).

(9) J. Folch-Pi, *Biochem. Rev.*, **17**, 147 (1948).

(10) D. W. Wooley, *J. Biol. Chem.*, **147**, 581 (1943).

(11) H. H. Hutt, T. Malkin, A. G. Poole and P. R. Watt, *Nature*, **165**, 314 (1950).

(12) W. Gordon Rose, THIS JOURNAL, **69**, 1384 (1947).

(13) I. R. Hunter, R. L. Roberts and E. B. Kester, *ibid.*, **70**, 3244 (1948).

(14) A. Grün and R. Limpächer, *Ber.*, **60**, 151 (1927).

(15) I. Kabashima, *ibid.*, **71**, 1071 (1939).

(16) The bis-α,α-[diacylglyceryl]-phenylphosphates (III, $R_1 \rightarrow R_4$) have also been obtained as by-products in the synthesis of α-lecithins and have been described by us on this occasion.[19] The bis-phosphatidic acids, which were obtained by the catalytic removal of the phenyl group from compounds III, $R_1 \rightarrow R_4$, as well as the higher poly-phosphatidic acids, are of particular interest as possible substitutes for cardiolipin in the serodiagnosis of syphilis. Attempts in this Laboratory to prepare phosphatidic and polyphosphatidic acids led to procedures which give the monophosphatidic acids [E. Baer, *J. Biol. Chem.*, **189**, 235 (1951)) and bis-phosphatidic acids in excellent yields. The synthesis of the bis-phosphatidic acids will be reported soon. A preliminary test of one of the bis-phosphatidic acids by Dr. R. H. Allen (Ottawa) disclosed that the substance possessed sufficient serological activity as a cardiolipin substitute to warrant further investigation. The work is being continued.

with ethyl acetate. Tne concurrent removal of the protective phenyl and carbobenzoxy groups[17] by catalytic hydrogenolysis in the presence of a mixture of platinum and palladium catalysts yields the desired L-, D- or DL-α-cephalin (V), respectively. The consecutive removal of the carbobenzoxy and phenyl groups, and the isolation of the intermediate diacylglycerylphenylphosphorylethanolamine were also investigated. This procedure, however, was found to be inferior to the one described herein.

As has been discussed more fully elsewhere,[18-20] mixed acid triglycerides can be assigned to either one of the two optical series depending on which part of the molecule serves as the stereochemical compound of reference. Thus an α-cephalin can be considered stereochemically either as a derivative of its diglyceride moiety (VII), making it a member of one optical series, or as a derivative of its glycerylphosphorylethanolamine moiety (GPE, VIII), making it a member of the opposite optical series. The choice, as in the case of the corresponding α-lecithins,[18,19] was influenced by biological as well as chemical considerations, and therefore the GPE moiety, which is the same in every α-cephalin, was chosen as the stereochemical compound of reference. Thus arbitrarily, but in conformity with the adopted usage in the α-lecithin series,[18,19] an α-cephalin is assigned the L-configuration if it contains L-α-GPE and D-configuration if it contains D-α-GPE.

From previous investigations in this Laboratory, it was known that the naturally occurring α-glycerophosphoric acid,[21] α-glycerylphosphorylcholine,[22,23] and distearoyl- and dipalmitoyl-α-lecithin[18,19] are members of the L-series. It seemed reasonable to assume that the natural α-cephalins

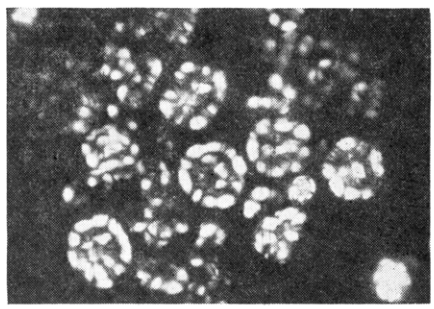
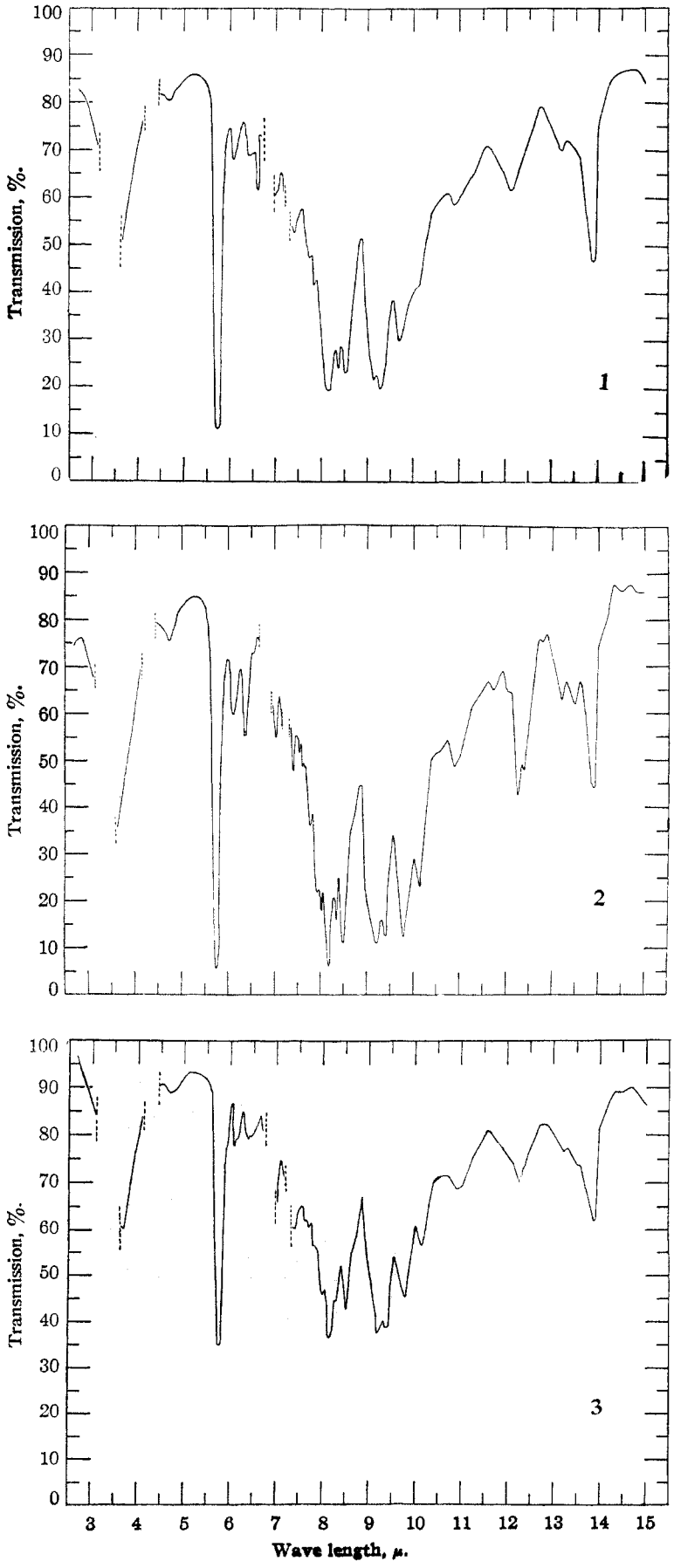


Fig. 1.—Photomicrograph of L-α-(dipalmitoyl)-cephalin: oil immersion objective (Leitz, N. A. 1.32); polarizing attachment; × 1200; Panatomic X; no filter.

(17) In contrast to Rose[12] we had no difficulty in removing the carbobenzoxy group by catalytic hydrogenolysis in the presence of palladium.

(18) E. Baer and M. Kates, *Science*, **109**, 31 (1949).
(19) E. Baer and M. Kates, THIS JOURNAL, **72**, 942 (1950).
(20) H. O. L. Fischer and E. Baer, *Chem. Revs.*, **29**, 287 (1941).
(21) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **128**, 491 (1939).
(22) G. Schmidt, B. Hershman and S. T. Thannhauser, *ibid.*, **161**, 523 (1945).
(23) E. Baer and M. Kates, THIS JOURNAL, **70**, 1394 (1948).



would be members of the same stereochemical series. Preference therefore was given to the synthesis of L-α-cephalins. This choice received further justification by the fact that Feulgen and Bersin's closely related plasmalogen, according to a recent study by Thannhauser, Boncoddo and Schmidt[24] is a derivative of the levorotatory glycerylphosphorylethanolamine (GPE) to which, by comparison with the synthetic levorotatory L-α-GPE,[25] we were able to assign the L-configuration.[26]

The following three cephalins, namely, L-α-(distearoyl)-cephalin (DSC), L-α-(dipalmitoyl)-cephalin (DPC) and L-α-(dimyristoyl)-cephalin (DMC) were prepared and were obtained in over-all yields of 51, 44 and 37%, respectively. Recrystallization from dioxane yielded the cephalins in the form of microscopic spherulites (see microphotograph) which exhibited birefringence under polarized light and gave distinct X-ray diffraction patterns.

The infrared spectra of the three α-cephalins were measured. The general absorption pattern, comprising a strong band at 5.73 μ, a strong band at 8.5 μ flanked by bands near 8 and 9 μ, and a band near 13.9 μ, resembles those reported by Shreve, Heether, Knight and Swern[27] for long-chain glycerides. The characteristic shift of the strong absorption band at 5.85 μ of the long-chain fatty acid spectra to 5.73 μ in the ester spectra[27] was also found in the cephalin spectra. One would predict from the similarity of the infrared spectra of the homologous straight chain fatty acids that the homologous cephalins should have virtually identical ab-

(24) S. T. Thannhauser, N. F. Boncoddo and G. Schmidt, *J. Biol. Chem.*, **188**, 423 (1951).

(25) The synthesis of L-α-glycerylphosphorylethanolamine has been accomplished in this Laboratory and will be reported soon.

(26) The fact that the naturally occurring α-lecithins,[19] α-cephalins, acetalphosphatides and their biological intermediates, namely, α-glycerylphosphorylcholine,[22] and α-glycerylphosphorylethanolamine,[15] without exception, have been identified as members of the L-series, suggests that these compounds arise biologically from a common source, the L-α-glycerophosphoric acid[21] originating in the carbohydrate cycle.

(27) O. D. Shreve, M. R. Heether, H. B. Knight and D. Swern, *Anal. Chem.*, **22**, 1498 (1950).

Fig. 2.—Infrared spectra of (1) L-α-(distearoyl)-cephalin, (2) L-α-(dipalmitoyl)-cephalin and (3) L-α-(dimyristoyl)-cephalin in nujol mulls (nujol: cephalin = 1:1 w./w.). Perkin–Elmer infrared spectrometer, Model 12 C, sodium chloride prism, spacer 0.05 mm. Thickness of cephalin layer approximately 0.03 mm.

sorption curves, and in fact the curves of the L-α-(dimyristoyl)- and L-α-(distearoyl)-cephalins are quite similar. The L-α-(dipalmitoyl)-cephalin shows, however, considerable differences in the region of 11.5–14 microns. The significance of these differences is difficult to assess. If impurities are present, their amounts are negligible since the analytical data are satisfactory. Neither are the differences attributable to the fatty acids, since the myristic, palmitic and stearic acid used by us, according to measurements by Dr. Norman K. Freeman,[28] have virtually identical infrared spectra.

It is interesting to note that the melting points, specific rotations and solubilities of DSC, DPC and DMC and of the ethanolamine salts of the corresponding α-phosphatidic acids[29] (Table I), are so similar that it would be difficult to distinguish the two classes of compounds by means of these physical constants alone. It is conceivable that the melting points observed are neither those of the phosphatidic acid ethanolamine esters nor of the ethanolamine salts, but of substances formed by both classes of compounds during the period of heating, involving either a migration of the phosphatidic acid moiety (O → N) or a loss of water This hypothesis is supported by the fact that acyl migrations from oxygen to nitrogen and in the reverse are fairly common[30] and that the choline esters and salts of phosphatidic acids, in which the methyl groups of the nitrogen prevent a similar migration or loss of water, indeed possess distinctly different melting points. The chloroform solutions of the synthetic cephalins when treated briefly with cold dilute sulfuric acid lost as expected only a few per cent. of their nitrogenous material, whereas the ethanolamine salts of the corresponding phosphatidic acids, when treated similarly, released all of their ethanolamine to the sulfuric acid.[29]

TABLE I

	M.p., °C., meniscus formation	$[\alpha]_D$	Solubility at 22–23° per 100 ml. of solution: Ethanol, mg.	Ether, mg.	Acetone, mg.
DS L-α-GPA					
Ethanolamine ester	172–173.5	+6.0°	8	<1	<1
Ethanolamine salt	172–173	+6.8	20	<1	<1
DP L-α-GPA					
Ethanolamine ester	172–175	+6.4	36	≷1	≷1
Ethanolamine salt	173–175	+7.7	40	<1	<1
DM L-α-GPA					
Ethanolamine ester	175–177	+6.7	80	<1	≷1
Ethanolamine salt	174–175	+8.8	150	<1	<1

It has been reported that the naturally occurring and partly unsaturated lecithins[31] and cephalins[32] have the capacity to render glucose, glycogen, sodium chloride and sodium sulfate soluble in ether. It was found that the fully saturated α-cephalins and α-lecithins are not soluble enough in ether at room temperature to have an effect on the solubility of glucose, glycogen, sodium chloride or sodium sulfate in this solvent. Moist ether (1% of water) solutions of the lecithins, however, take up small amounts of sodium chloride; so do the chloroform solutions of the α-cephalins and α-lecithins, which in addition dissolve also glucose, and glucose and sodium sulfate, respectively.

In the course of his study on the acid-resistant staining properties of tubercle bacilli, Koganei[33,34] was able to show that, contrary to views held at the time, the cephalins and not the fats, fatty acids or waxes possess the receptive staining capacity, and that the degree of saturation of the fatty acid radicals has little influence on the staining character of the cephalins. The staining property is also shown by the ether solutions of cephalins, which, according to Koganei, even in great dilution are capable of taking up basic dyestuffs, such as fuchsin, methyl blue, gentiana violet or Bismark brown. Since Koganei's experiments were carried out with a natural cephalin of doubtful purity they were repeated by us using the pure synthetic L-α-(dimyristoyl)-cephalin. Our experiments confirmed the staining of cephalin in ether solution by fuchsin, gentiana violet and Bismark brown but not by methyl blue. The α-lecithins, as tests with the synthetic material showed, possess similar staining properties.

The quantitative study of the acid and alkaline hydrolysis of α-glycerylphosphorylcholine[35] and of α-lecithins[36] has revealed that the hydrolysis of these substances is accompanied by a reversible migration of phosphoric acid. As a result the evidence which has been accepted as proof for the natural occurrence of β-lecithins is now considered fallacious. The data presented in support of the simultaneous natural occurrence of α- and β-cephalins,[37–42] deduced from similar evidence, suggest strongly that the hydrolysis of cephalins by acid or alkali likewise is accompanied by a reversible phosphoric acid migration. An investigation of the chemical hydrolysis of α-cephalins and of the α-GPE-moiety, using the synthetic materials, is in progress in this Laboratory.

The procedure described in this communication makes available for the first time pure individual α-cephalins of known constitution and configuration.

Experimental

L-α-(Distearoyl)-cephalin. Distearoyl-L-α-glycerylphenylphosphoryl-(N-carbobenzoxy)-ethanolamine (IV R_1) (a) Phosphorylation.—Into a dry 250-ml. round-bottomed three necked flask equipped with an oil-sealed and motor-driven stirrer, calcium chloride tube and dropping funnel, were placed 0.02 mole (4.22 g.) of freshly prepared and carefully fractionated monophenylphosphoryl dichloride, 0.04 mole (3.22 ml.) of anhydrous pyridine, and 0.1 mole (8 ml.) of anhydrous and ethanol-free chloroform. The

(28) University of California, Division of Medical Physics, Donner Laboratory, Berkeley, California. Personal communication.

(29) See also E. Baer, *J. Biol. Chem.*, **189**, 235 (1951), Table I.

(30) See H. Meyer, "Lehrbuch der Organisch-Chemischen Methodik," 4th edition, Julius Springer, 1922, Vol. 1, p. 925, references 9–11.

(31) H. J. Bing, *Scand. Arch. Physiol.*, **11**, 166 (1901).

(32) A. Frank, *Biochem. Z.*, **50**, 273 (1913).

(33) R. Koganei, *J. Biochem.*, **1**, 353 (1922).

(34) R. Koganei, *ibid.*, **2**, 495 (1923).

(35) E. Baer and M. Kates, *J. Biol. Chem.*, **175**, 79 (1948).

(36) E. Baer and M. Kates, *ibid.*, **185**, 615 (1950).

(37) B. Suzuki and U. Nishimoto, *Proc. Imp. Acad. Japan*, **6**, 262 (1930).

(38) B. Suzuki and Y. Yokoyama, *ibid.*, **6**, 341 (1930).

(39) Y. Yokoyama and B. Suzuki, *ibid.*, **8**, 183 (1932).

(40) Y. Yokoyama, *ibid.*, **9**, 582 (1934).

(41) U. Nishimoto, *ibid.*, **10**, 578 (1934).

(42) C. F. Burmaster, *J. Biol. Chem.*, **165**, 565 (1946).

flask was immersed in a water-bath which was kept at 8° and a solution of 0.02 mole (12.5 g.) of D-α,β-distearin[43] in 1.25 moles (100 ml.) of anhydrous chloroform was added, dropwise and in the course of 25 minutes, to the vigorously stirred phosphorylation mixture. Fifteen minutes after the last of the distearin had been added, the cold-bath was removed and the reaction mixture was kept at room temperature (24°) for one hour. At the end of this period there was added a solution of 0.02 mole (3.9 g.) of carbobenzoxy-ethanolamine[12] (freshly recrystallized from ether) in 0.08 mole (6.5 ml.) of anhydrous pyridine,[44] and the mixture, protected from moisture, was allowed to stand for 20 hours at room temperature.

(b) **Isolation of the Phosphorylation Product.**—The reaction mixture was brought to dryness under reduced pressure at a water-bath temperature not exceeding 35°. The solid residue was freed of pyridine as thoroughly as possible in a high vacuum (0.02 mm.) and was then triturated with four 80-ml. portions of boiling petroleum ether (b.p. 35–60°). Solid and extracts were separated each time by centrifugation. The combined extracts, which had a tendency to become turbid, were brought to dryness under reduced pressure. The residue weighing 12.6 g. was extracted at 35° with three 60-ml. portions of anhydrous ethyl acetate[45] and the combined extracts were allowed to stand undisturbed overnight at room temperature (22–25°). The small amount of precipitate which had formed was removed by centrifugation and the decanted supernatant solution was brought to dryness under reduced pressure. In order to obtain a powdery substance the residue was dissolved in petroleum ether (b.p. 35–60°), the solution was cleared by centrifugation and the supernatant was brought to dryness *in vacuo* at a bath temperature of 10–15°. The substance was kept *in vacuo* (0.02 mm.) until constant weight was reached. If the material was still sticky the treatment with petroleum ether was repeated. The distearoyl-L-α-glyceryl-phenylphosphoryl-(N-carbobenzoxy)-ethanolamine (IV R_1) was obtained in a yield of 54.3% (10.4 g.) of the theory based on distearin. The substance started to sinter at 48° and melted from 51–52°. It was very soluble in chloroform, fairly soluble in ethyl acetate, ether, or warm petroleum ether (b.p. 35–60°) and insoluble in water; $[\alpha]^{27}D$ +2.1° in dry and ethanol-free chloroform (*c* 10); *M*D +20.1°. *Anal.*[46] Calcd. for $C_{55}H_{92}O_{10}NP$ (958.3): C, 68.93; H, 9.68; N, 1.46; P, 3.24. Found: C, 69.11; H, 9.66; N, 1.48; P, 3.30. The phenyl esters IV $R_1 \rightarrow R_3$ on standing, gradually liberated phenol which was detected by its characteristic odor.

Distearoyl-L-α-glycerylphosphorylethanolamine (V R_1). **Simultaneous Catalytic Removal of the Protective Groups.**—A solution of 10.85 millimoles (10.4 g.) of distearoyl-L-α-glycerylphenylphosphoryl-(carbobenzoxy)-ethanolamine in 6 moles (343 ml.) of glacial acetic acid (C.P. reagent, Grasselli Brand) together with 30 milliatoms (3.2 g.) of palladium catalyst[47] and 15 millimoles (3.68 g.) of platinum dioxide[48] (Adams catalyst) in a hydrogenation vessel of glass (1-l. capacity) were shaken vigorously in an atmosphere of hydrogen at an initial pressure of approximately 50 cm. of water. At the end of 45 minutes the absorption of hydrogen had ceased and 2590 ml. (23°, 750 mm.) of hydrogen had been consumed.[49] After replacing the hydrogen with nitrogen, the precipitate was brought into solution by adding 250 ml. of chloroform and the catalyst was removed by centrifugation. The catalyst was washed with a small volume of a mixture of acetic acid–chloroform (1:1). The combined solutions were brought to dryness under reduced pressure at a water-bath temperature of 30° and the solid residue was redissolved in 35 ml. of chloroform. The chloroform solution was freed of traces of catalyst by centrifugation, and the cephalin was precipitated by the addition of 270 ml. of acetone. The mixture was centrifuged and the solid, after washing thoroughly with anhydrous and peroxide-free ether, was dried *in vacuo* over phosphorus pentoxide to constant weight. The yield of analytically pure L-α-(distearoyl)-cephalin was 7.64 g. (94.1%). Over-all yield based on distearin 51.1%. The cephalin started to sinter at approximately 83° and melted with meniscus formation from 173–175° (rate of heating 3° per min. from 150°); $[\alpha]_D^{24}$ + 6.0°[50] in a mixture of dry and ethanol-free chloroform and glacial acetic acid (9:1v./v.), (*c* 4.4); *M*D +44.5°. *Anal.* Calcd. for $C_{41}H_{82}O_8NP$ (748.1): C, 65.82; H, 11.04; N, 1.87; P, 4.14; glycerol, 12.3. Found: C, 65.79; H, 10.80; N, 1.89 (Dumas), 1.76 (Kjeldahl); P, 4.16; glycerol,[51] 12.3.

L-α-(Dipalmitoyl)-cephalin.—The two-stage phosphorylation (a) of D-α,β-dipalmitin, and the isolation (b) of the dipalmitoyl-L-α-glycerylphenylphosphoryl-(N-carbobenzoxy)-ethanolamine, were carried out as described for distearin, using the same molecular ratios for reagents and solvents, but reducing in step (b) the volume of petroleum ether and of ethyl acetate to one-half and one-third, respectively, of those reported for the extraction and purification of the distearin compound (IV R_1) and carrying out the extraction with ethyl acetate at 24° (water-bath).

Dipalmitoyl-L-α-glycerylphenylphosphoryl-(N-carbobenzoxy)-ethanolamine (IV R_2).—0.02 mole (11.38 g.) of D-α,β-dipalmitin[43] yielded 8.87 g. of compound IV R_2, corresponding to 49.2% of theoretical yield based on dipalmitin. The substance started to sinter at 42° and melted with meniscus formation from 43–44°; $[\alpha]^{26}D$ +2.3° in dry and ethanol-free chloroform, (*c* 9.2), *M*D +21.0. *Anal.* Calcd. for $C_{61}H_{84}O_{10}NP$ (902.5): C, 67.89; H, 9.38; N, 1.55; P, 3.44. Found: C, 67.95; H, 9.41; N, 1.53 (Dumas); P, 3.52.

At room temperature substance IV R_2 was very soluble in chloroform, fairly soluble in ethyl acetate, acetone, 95% ethanol or ether, slightly soluble in acetic acid or low boiling petroleum ether (fairly soluble in warm petroleum ether), and insoluble in water.

Dipalmitoyl-L-α-glycerylphosphorylethanolamine (V R_2). —The reductive cleavage of IV R_2 was carried out as described for the higher homolog using the same molecular ratios of substituted cephalin to palladium and platinum dioxide, but reducing the volume of glacial acetic acid to approximately three-fourths of that used in the cleavage of IV R_1. The time required to complete the reductive cleavage was approximately one hour. 8.87 g. of compound IV R_2 yielded 7.27 g. of already fairly pure L-α-(dipalmitoyl)-cephalin (V R_2). For further purification the substance was precipitated from 12 ml. of chloroform by the addition of 125 ml. of acetone and by cooling to 12°. The cephalin, after washing thoroughly with peroxide-free ether and drying *in vacuo* (0.02 mm.) over solid sodium hydroxide, weighed 6.14 g., which corresponded to a yield of 90.6% of theory for the reductive cleavage, and an over-all yield of 44.4% of theory based on dipalmitin. The cephalin started to sinter at approximately 88° and melted with meniscus formation from 172.5–175° (rate of heating 3° per min. from 150° on) $[\alpha]^{26}D$ +6.4° in dry and ethanol-free chloroform (*c* 7.8); *M*D +43.5. *Anal.* Calcd. for

(43) J. C. Sowden and H. O. L. Fischer, THIS JOURNAL, **63**, 3244 (1941).

(44) Pyridine of good commercial grade was refluxed over barium oxide and distilled with the exclusion of moisture. The chloroform was freed of alcohol immediately before use by distillation from phosphorus pentoxide.

(45) The ethyl acetate-insoluble part of the residue, weighing 2.22 g., was fairly pure bis-α,α-[distearoylglyceryl]-phenylphosphate (III R_1), m.p. 79–80.5°. *Anal.* Calcd. for $C_{84}H_{165}O_{12}P$ (1387): P, 2.24. Found: P, 2.41. The phosphorylation of D-α,β-dipalmitin and D-α,β-dimyristin yields the corresponding bis-compounds (III R_2, III R_3).

(46) The molecular weights and percentage compositions throughout this paper were calculated using the atomic weights 12.01, 1.008 and 31.02 for carbon, hydrogen and phosphorus, respectively.

(47) J. Tausz and N. v. Putnoky, *Ber.*, **52**, 1573 (1919).

(48) No attempts were made to establish the optimal ratio of the catalysts to phenyl-carbobenzoxycephalin. It is quite possible that the amounts of both catalysts can be reduced substantially.

(49) Because of the evolution of carbon dioxide during the cleavage it is impossible to measure accurately the uptake of hydrogen. In several experiments the catalytic cleavage was carried out in a current of hydrogen and the excess gas was passed through aqueous baryta. The gravimetrically determined barium carbonate did not amount to more than 70% of the theoretical.

(50) This rotation is identical with that reported by Levene and West[4] for the distearoyl cephalin isolated from a hydrogenated mixture of cephalins and lecithins [egg yolk]. Thus, if one assumes that the distearoyl cephalin is the compound claimed, it and its unsaturated precursor are established as members of the L-series. This is the first instance in which it has been possible to classify sterochemically a natural cephalin.

(51) The cephalin was boiled under reflux with hydriodic acid and the propyl iodide was determined according to F. Vieböck and A. Schwappach (*Ber.*, **63**, 2818 (1930)).

$C_{37}H_{74}O_8NP$ (692): C, 64.21; H, 10.78; N, 2.02; P, 4.48. Found: C, 64.15; H, 10.66; N, 1.85 (Dumas); P, 4.50.

L-α-(Dimyristoyl)-cephalin.—The two-stage phosphorylation of D-α,β-dimyristin was carried out as described for distearin, using the same molecular ratios for the reagents, but reducing in step (a) the amount of chloroform by one quarter and the temperature of phosphorylation to +4°. After the phosphorylation was completed the mixture was brought to dryness and as much as possible of the pyridine was removed *in vacuo* (0.1–0.3 mm.). To obtain the residue as a solid, it was distributed in 70 ml. of warm (40°) petroleum ether and the mixture was brought to dryness under reduced pressure (bath 25°). This procedure was repeated twice. The dimyristoyl-L-α-glycerylphenylphosphoryl-(N-carbobenzoxy)-ethanolamine (IV R_3) was isolated and purified as described for the corresponding distearoyl compound (b) but using only one fourth of the volumes of petroleum ether and ethyl acetate, and carrying out the extraction with ethyl acetate at 24°.

Dimyristoyl-L-α-glycerylphenylphosphoryl-(N-carbobenzoxy)-ethanolamine (IV R_3).—0.02 mole (10.25 g.) of D-α,β-dimyristin[19] yields 7.95 g. of IV R_3, corresponding to 46.9% of the theory based on dimyristin. The substance sintered at 31–33° and melted with meniscus formation at 35–36°; $[\alpha]^{26}D$ + 2.5° in ethanol-free chloroform (*c* 10), M_D +21.2°. *Anal.* Calcd. for $C_{47}H_{75}O_{10}NP$ (846.1): C, 66.71; H, 9.06; N, 1.65; P, 3.66. Found: C, 66.67; H, 9.10; N, 1.63; P, 3.67.

Dimyristoyl-L-α-glycerylphosphorylethanolamine (V R_3).—The reductive cleavage of IV R_3 (7.95 g.) was carried out as described for the IV R_1, using the same molecular ratios of substituted cephalin to palladium and platinum dioxide, but reducing the volume of glacial acetic acid to approx. one-half of that used in the cleavage of IV R_1. The reductive cleavage was completed in approx. 1 hour. After the removal of palladium and platinum the acetic acid solution was evaporated to dryness *in vacuo* (bath 35°), the residue was dissolved in 17 ml. of chloroform, the chloroform solution was freed of traces of catalyst by centrifugation, and the cephalin was precipitated by the addition of 210 ml. of acetone and cooling the mixture to +12°. The cephalin was centrifuged off, washed with peroxide-free ether and freed of solvents *in vacuo*. To remove small amounts of impurities with higher phosphorus content, the cephalin was triturated successively with three 100-ml. portions of distilled water and one 100-ml. portion of acetone. The solid was dried *in vacuo* (0.02 mm.) over phosphorus pentoxide to constant weight. The yield of analytically pure L-α-(dimyristoyl)-cephalin (V R_3) was 4.7 g. (78.8%). The over-all yield based on dimyristin was 37%. The cephalin started to sinter at approx. 86° and melted with meniscus formation and discoloration from 175–177° (rate of heating 2–3° per min. from 150° on); $[\alpha]^{26}D$ +6.7° in dry and ethanol-free chloroform (*c* 8.4); M_D +42.5°. *Anal.* Calcd. for $C_{83}H_{66}O_8NP$ (635.9): C, 62.33; H, 10.46; N, 2.2; P, 4.88. Found: C, 62.16; H, 10.51; N, 2.1; P, 4.85.

The approximate solubilities of synthetic L-α-cephalins in various solvents at 20° were determined. The cephalins were found to be insoluble (≷ 1 mg./100 ml. of dry solvent) in acetone, ether, petroleum ether and ethyl acetate; moderately soluble (20–100 mg./100 ml. of dry solvent) in ethanol, pyridine, benzene and carbon tetrachloride, and readily soluble (> 1 g./100 ml. of solvent) in chloroform. The solubility of the cephalins in the same solvent increases with decreasing length of the fatty acid chain. As might have been anticipated, the three synthetic α-cephalins are considerably less soluble in alcohol at room temperature (8 mg. DSC, 36 mg. DPC and 80 mg. DMC per 100 ml.) than the corresponding α-lecithins (0.8 g. DSL, 1.5 g. DPL and > 15 g. DML per 100 ml.). It is of interest to note in this connection that Folch has reported that the phosphatidyl ethanolamine isolated by him is readily soluble in alcohol. It is possible that the greater solubility of the natural substance is explained by the degree of unsaturation of its fatty acids.

The three synthetic cephalins after recrystallization from warm dioxane gave distinct X-ray diffraction patterns; Debye-Scherrer powder camera (114.5 mm.), radiation CuKα (λ 1.54K_x) nickel filter. Actual diameters in centimeters as measured on the original photographs and visually estimated relative intensities (in parentheses): Distearoyl-L-α-cephalin 3.93 (0.3), 4.31 (1.0), 4.66 (0.5), 5.62 (0.1), 8.06 (0.1), 9.17 (0.3), 10.65 (0.1); dipalmitoyl-L-α-cephalin 3.83 (0.4), 4.30 (1.0), 4.76 (0.4), 5.66 (0.4), 6.39 (0.1), 7.23 (0.1), 8.10 (0.3), 10.02 (0.1); dimyristoyl-L-α-cephalin 3.03 (0.3), 3.46 (0.3), 3.74 (0.5), 4.34 (1.0), 4.65 (0.7), 5.98 (0.2), 7.41 (0.1), 8.00 (0.1), 9.21 (0.3).

The synthetic α-cephalins and α-lecithins, which were also tested, gave the Casanova color reaction.[52] When an intimate mixture of equal volumes of ether, containing the synthetic cephalin or lecithin, and an aqueous solution of ammonium molybdate was run carefully upon concentrated sulfuric acid, a deep blue color gradually formed at the interphase.

The Solubility of Glucose, Glycogen, Sodium Chloride or Sodium Sulfate in Ether or Chloroform in the Presence of a Cephalin or Lecithin.—The qualitative solubility tests were carried out as follows. Three 10-ml. flasks were charged each with 5 ml. of solvent (anhydrous ether, ether containing 1% of water or anhydrous and ethanol-free chloroform). To the first flask was added approx. 50 ml. of the dimyristoylcephalin or dimyristoyllecithin, to the second flask approx. 50 mg. of the finely powdered substance to be tested for its solubility and to the third flask approx. 50 mg. of both, the phosphatide and the substance to be tested. After being shaken at room temperature (20–22°) for a period of 15 minutes, the mixtures were centrifuged, and the decanted and filtered supernatants were brought to dryness in a current of warm air.

To test for glucose the residues were dissolved or suspended each in 5 ml. of water, and the aqueous solutions or suspensions, after adding 5 drops of Fehling solution, were heated in a boiling water-bath for a period of 10 minutes. For comparison a test-tube containing 5 ml. of water, approx. 50 mg. of glucose and 5 drops of Fehling solution was placed in the water-bath.

The tests for the presence of sodium chloride or sodium sulfate were carried out by dissolving or suspending the air-dried residues in 5 ml. of 1 *N* nitric acid and testing the filtrates in the usual manner for chlorine or sulfate ion.

To test for glycogen the solutions or suspensions of the air-dried residues in 0.5 *N* hydrochloric acid, were heated in a boiling water-bath for 15 min., and the filtrates were tested for glucose with Fehling solution. The results are summarized in Table II.

TABLE II

	Anhydrous ether	Ether + 1% water	Chloroform
Dimyristoylcephalin			
Glucose	−	−	++
Glycogen			−
Sodium chloride	−	−	+
Sodium sulfate (anhydrous)	−		−
Dimyristoyllecithin			
Glucose	−	−	++
Glycogen			−
Sodium chloride	−	+	+
Sodium sulfate (anhydrous)	(±)		+

(−) insoluble, (+) just perceptibly soluble and (++) distinctly soluble.

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(52) See "The Merck Index," 5th Ed., Merck and Co., Inc., Rahway, N. J., p. 668, reaction 607.